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Factors influencing stem cell mobilization in patients with hematologic malignancies and solid tumors

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Summary

Mobilized peripheral blood stem cells (PBSC) have become the main source for autologous and allogeneic hemopoietic stem cells transplantation (HSCT) following myeloablative therapy in patients with hematological malignancies or solid tumors. Classical strategies for PBSC mobilization include administration of granulocyte colony-stimulating factor (G-CSF) alone or in combination with other agents or myelosuppressive chemotherapy. PBSC mobilization and collection have been optimized in numerous clinical trials, but a proportion of patients fail to mobilize. The aim of the study was to establish the influence of diagnosis, sex, age, number of previous courses of chemotherapy, mobilization regimen, and bone marrow (BM) involvement on the outcome of peripheral blood stem cell mobilization.

Patients and methods. 121 patients with hematological malignancies and solid tumors were included in the study (Hodgkin's lymphoma (HD) (n=24), non-Hodgkin lymphomas (NHL) (n=29), multiple myeloma (MM) (n=32), acute leukemias (AL) (n=15), and solid tumors (ST) (n=21)). 100 patients (82%) were mobilized with G-CSF, and a combination of chemotherapy and G-CSF was used in 21 patients (18%). 57 patients (49%) received more than six courses of chemotherapy and 74 (51%) less than six respectively. The criterion for adequate mobilization was a score of at least 2.0 x 10^6 CD34+ cells/kg of body weight.

Results. In our trial there was no correlation between PBSC yield and the patient's diagnosis, age, or gender. BM involvement does not seem to be an independent factor, with significant adverse influence on PBSC mobilization (p=0.78). Stem cell yield was significantly higher in those patients who received fewer than six courses of chemotherapy (10.0 ± 2.2 x 10^6 CD^{34+}/kg against 5.5 ± 1.7 x 10^6 CD^{34+}/kg (p=0.006)). A better outcome was seen in patients mobilized with chemotherapy plus G-CSF than with G-CSF alone (8.12 ± 1.12 x 10^6 CD^{34+}/kg against 6.9 ± 0.9 x 10^6 CD^{34+}/kg (p= 0.008)).

Conclusions. Diagnosis, age, sex, and bone marrow involvement does not influence the outcome of stem cell mobilization. Better stem cell yield was seen in patients who received fewer than six courses of chemotherapy, and in patients mobilized with cytokines combined with chemotherapy than cytokines alone.

Keywords: stem cell mobilization, peripheral blood, G-CSF, chemotherapy, combined protocol, transplantation outcome

1. Introduction

In the early 1990s, PBSC replaced bone marrow as the preferred source of stem cells because of the relative ease of collection, and the faster hematological recovery when compared with bone marrow transplantation (BMT), which in turn leads to fewer complications, and lowers the costs of the procedure [1]. Small but distinct numbers of HSCs are in circulating blood even in the normal steady state, and recruitment of HSCs to peripheral blood following chemotherapy and/or treatment with cytokines, which is termed peripheral blood stem cell (PBSC) mobilization, is necessary and widely used in clinical practice. Currently, high-dose chemotherapy with autologous stem cell transplantation is increasingly used in a wide range of hematological and solid malignancies [2,3]. Successful autologous peripheral blood stem cell transplantation (ASCT) depends on the infusion of an adequate number of peripheral blood progenitor cells (PBPC). For rapid hematological recovery and durable engraftment, an infusion of a minimum of 2.0 x 10^6 CD34+ cells per kg of a patient's body weight (/kg) is required. However, a CD34+ cell dose of 5.0 x 10^6/kg or greater may be optimal and preferred, as virtually all patients infused with this dose experience hematological recovery within two weeks. The identification of risk factors of poor PBPC collection in patients with hematologic or solid malignancies is of clinical relevance for therapeutic decisions. In recent years, some of the underlying physiology has been elucidated, leading to the development of new mobilization strategies. Expression of the G-CSF receptor on stem cells is not required for mobilization. G-CSF or myelosuppressive therapy acts via secondary pathways, including the chemokine stromal-derived factor-1 and its receptor CXCR4 [4]. A multitude of agents are being developed and tested to be used alone or in combination with G-CSF or chemotherapy in order to successfully remobilize poor mobilizers, reduce the number of apheresis sessions and/or the amount of G-CSF required for successful PBSC mobilization. Furthermore, the combinations and new agents are used to improve the hematopoietic recovery after PBSC transplantation [5]. New chemokine receptor agonists lead to a rapid and substantial PBSC mobilization. A pegylated G-CSF (pegfilgrastim) now has become available. The administration of a single dose of 30–300µg/kg of pegfilgrastim resulted in a significant mobilization of CD34+ cells in tumor patients treated with chemotherapy [6,7]. The combination of stem cell factor (SCF), a cytokine acting on early stem cells, with G-CSF resulted in improved mobilization in conjunction with reduced apheresis numbers in multiple myeloma patients [8-10]. In 44% of heavily pretreated patients with Hodgkin's disease or non-Hodgkin's lymphoma, it resulted in a successful mobilization (5 x 10^6 CD34+ cells/kg bw) compared with only 17% in the G-CSF group [1]. Significant progress has been made in understanding the mechanisms of PBSC mobilization. This has led to the development of new agents that are already being tested in clinical trials. AMD-3100, a small synthetic molecule and a partial CXCR4 agonist, is the most promising compound in this series [11,12,10].

We have retrospectively analyzed data from 121 consecutive patients with malignancies who had undergone PBPC harvests to define the predictive factors that affect the ability to collect an adequate number of PBPC for AHSCT, and the time to reach the target PBSC collection.

2. Materials and methods

2.1. Patients

121 patients undergoing autologous stem cell transplantation between February 2000 and December 2005 were in our center. The patients' diagnoses, gender, median age, median number of lines of prior chemotherapy, and disease status at transplantation, and the percentage of patients who received prior radiotherapy are listed in Table 1. Patients were enrolled in the study if they were eligible for autologous stem cell transplantation. All patients signed informed concern approved by our local ethics committee.

Number of patients	**N = 121**
Median age at mobilization (years)	43 (18–67)
Gender	
F	52 (46.6%)
M	69 (53.4%)
Diagnosis	
Multiple myeloma	32 (38.4%)
Hodgkin's disease	29 (18.6%)
Non-Hodgkin's lymphomas	24 (27.7%)
Acute leukemias	15 (9.1%)
Solid tumors	21 (6.2%)
Complete remission at mobilization	
Yes	84 (82.08%)
No	37 (17.91%)
Bone Marrow involvement at mobilization	
Yes	17 (3.25%)
No	104 (96.75%)
Median number of previous chemotherapy courses	8 (1–18)
Previous radiotherapy	
Yes	26 (28.3%)
No	95 (71.7%)
Mobilization regimen	
G-CSF	100
G-CSF+ chemotherapy	21
Median of leukapheresis procedures/patient	2 (1–3)
Median of CD34+ cells collected per patient (x 106/kg of patient weight)	6.5 (1.2–24.6)

Table 1. Patient characteristics

2.2. PBSC mobilization, harvest and CD34+ cell quantification

100 patients were mobilized with G-CSF alone (Neupogene, Roche, USA) with a dosage of 10µg/kg subcutaneously twice daily. 21 patients received high-dose cyclophosphamide at 4,000 mg/m^2. Others were mobilized with various disease-specific chemotherapy regimens that were chosen for both their efficacy against the patients' disease and their ability to induce a WBC rebound following marrow aplasia. The most common chemotherapy regimens were BEACOPP (cyclophospamide, etoposide, doxorubicine, procarbazine, vincristine, bleomycine, prednisolone), dexaBEAM (carmustine, cytarabine, etoposide, dexamethasone, melphalane), and DHAP (cytarabine, cisplatin, dexamethasone).

For patients mobilized with cyclophosphamide or other chemotherapy, the first dose of G-CSF was given subcutaneously at a dose of 5 mg/kg/day from the day +5 after chemotherapy had ended, and continued until the day before the last leukapheresis. In all cases the criterion for adequate mobilization was a score of at least 2.0 x 10^6 CD34+ cells/kg of body weight.

2.3 Collection and cryopreservation of PBSCs

PBPC were collected with a continuous-flow blood cell separator (Fenwal CS3000 plus, Baxter healthcare, Deerfield, IL, USA). Each apheresis procedure was performed for approximately 2 to 4 hours, processing 10–14 l of whole blood volume. The total MNC count and CD34+ count of the leukapheresis product was monitored daily following each collection. When the white blood count (WBC) had risen to greater than 1000 cells/µl, the daily peripheral CD34+ count was determined. PBSC collection began when peripheral CD34+ numbers were ≥10 cells/µl. The goal was to collect ≥2.0 x 10^6 cells CD34+cells per kg of patient weight, which was the criterion for adequate PBPC collection in our study. However, PBPC harvests were discontinued after at least two days from the initiation of leukapheresis when it seemed that they were not likely to yield ≤0.2 x 10^6 CD34+ cells/kg per day. The cells were cryopreserved according to standard procedure using a Planer programme freezer (UK).

2.4 CD34+ cell enumeration

CD34+ cells were enumerated in the peripheral blood and leukapheresis product by using flow cytometry. Briefly, 5 ml aliquots of the samples were incubated for 20 min at room temperature with phycoerythrin (PE)-conjugated monoclonal antibody (moAb) anti-CD34, and fluorescein isothiocyanate (FITC)-conjugated moAb anti-CD45. After incubation, red cells were lysed and washed in phosphate-buffered saline. Cells were analyzed by flow cytometer FACScalibur (Becton Dickinson, San Jose, CA, USA). 100,000 cells were analyzed for each sample.

2.5 Response to mobilization

Patients were divided into "good" and "poor" mobilizers. Good mobilizers were defined when ≥2.0 x 10^6 cells of CD34+ per kg of patient weight could be collected in a maximum of three leukapheresis procedures. Patients who were poor mobilizers needed more than three leukapheresis procedures to collect a number ≤2.0 x 10^6 cells CD34+ per kg of patient weight, and when a minimum of 10 CD34+ cells/µl was not reached in the peripheral blood after mobilization.

2.6. Statistics

The results of the study were analyzed with SPSS 13.0, using parametric and non-parametric methods.

3. Results

We analyzed 316 mobilization cycles in 121 patients. 104 patients (85%) achieved at least 2.0 x 10^6 CD 34+ cells/kg of body weight. 17 patients (15%) were poor mobilizers. Data of all patients are summarized in Table 2.

	Number of CD34+ x 10^6 cells/kg body weight
Age at mobilization (years)	
> 50	4.7±2.1
< 50	5.3±1.7, p=0.76
Gender	
Female	6.8±2.5
Male	5.8± 2.6, p=0.89
Bone Marrow involvement at mobilization	
Yes	7.2±3.8
No	9.4±1.6, p=0.78
Complete remission at mobilization	
Yes	6.7±2.2
No	5.6±3.1, p=0.23
Median number of previous chemotherapy courses	
> 6	3.2±2.0
< 6	7.9±4.3, p≤0.0001
Previous radiotherapy	
Yes	5.9±3.6
No	6.4±3.4, p=0.5
Mobilization regimen	
G-CSF	6.7±5.2
G-CSF+ chemotherapy	14.7±3.9, p≤0.008

Table 2. Results of HSC mobilization

3.1 Influence of diagnosis, gender, and age on PBSC mobilization

In our trial there was no correlation between PBSC yield and the patient's age. The same was true of gender. There was also no influence on PBSC mobilization kinetics due to their diagnosis.

3.2 Influence of bone marrow involvement

The influence of marrow involvement on PBPC mobilization was studied in patients with multiple myeloma and non-Hodgkin lymphomas. We didn't observe a significant difference in blood levels of CD34+ cells or apheresis yields between patients who didn't reach complete response of the disease after previous chemotherapy and who had more or less than 50% marrow infiltration at the time of aperesis (data not shown).

3.3 Influence of previous cytotoxic chemo- and radiotherapy

A poor mobilization of progenitor cells was observed in patients who had been intensively treated with chemotherapy. Therefore, in those patients who had received a greater number of different chemotherapy regimens prior to harvest, the number of CD34+ cells collected was lower. This was observed when comparing patients who had received 1–5 pre-mobilization cycles of chemotherapy, and patients with more than 5 cycles ($p<0.01$). Additionally, lower progenitor cell yields were obtained in patients who had received melpalan-containing regimens (miniBEAM, DexaBEAM, melphalan plus prednisolone), than in patients without these charac-

teristics. By contrast, prior radiotherapy wasn't a significant factor affecting PBPC yield (p=0.5). In previously irradiated patients the mean number of CD34+ cells per apheresis was 5.9 x 10^6/kg, while in those who did not receive radiation therapy the mean number was 6.4 x 10^6/kg.

3.4 Influence of mobilization regimen

We have studied the effects of G-CSF on chemotherapy-induced stem cell mobilization on peripheral blood and on the yield of stem cell harvest in patients with hematological malignancies. The results of mobilization were much better when using a combination regimen of G-CSF and chemotherapy, than G-CSF alone (p=0.008) (see Table 2).

Conclusion

The number of CD34+ cells transplanted can help predict the speed of engraftment after myeloablative therapy [13]. A subset of patients, often heavily pretreated, do not achieve satisfactory PBSC mobilization [14]. Many studies proved that prior radiotherapy and chemotherapy with alkylating agents (melphalan, carmustine) adversely affect mobilization [15,16]. Melphalan is particularly stem-cell toxic, and even a low dose given orally before mobilization results in reduced CD34+ cell mobilization [4]. The most negative predictive factor for progenitor cell yield in our study was the extent of prior chemotherapy, as has been described for chemotherapy mobilization [17,18]. Pretreatment with more than six cycles of chemotherapy resulted in a significantly lower cell harvest than pretreatment with fewer than six cycles ($p \leq 0.0001$). We observed that in patients who received more than six cycles of chemotherapy pCD34+ cell count was achieved later and showed a lower maximal height of pCD34+ cells/l PB. This finding agrees with previous studies supporting that PBSC collection should be performed early in the course of disease to avoid CT-induced stem cell damage [4,14,18]. Further studies of new mobilization agents (pegylated G-CSF, CXCR-inhibitors) and their combinations are needed to improve the result of stem cell mobilization and to solve the problem of so-called "poor" mobilizers.

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Оценка факторов, влияющих на эффективность мобилизации гемопоэтических стволовых клеток периферической крови у пациентов с гемобластозами и солидными опухолями

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Резюме

Целью мобилизации ГСК является получение не менее 2×10^6 CD34+ клеток на килограмм массы тела реципиента, что принято считать нижним уровнем, позволяющим достичь быстрого и устойчивого приживления трансплантата. Опыт использования Г-КСФ в качестве мобилизующего агента позволил разработать стандартные схемы стимуляции. Тем не менее, у определенной части пациентов при планировании аутологичной трансплантации в ходе стандартной процедуры мобилизации не удается получить достаточного количества ГСК. Плохой ответ или отсутствие ответа на мобилизацию требует проведения последующей ремобилизации и/или дополнительных процедур афереза, что негативно сказывается на состоянии пациента, а также приводит к увеличению экономических затрат.

Значительное число пациентов, плохо отвечающих на мобилизацию, делает необходимым анализ факторов, влияющих на эффективность данного процесса. В исследовании проведен анализ клинико-лабораторных данных с целью выявления факторов, оказывающих влияние на результат мобилизации.

Ключевые слова: стволовые клетки, мобилизация, периферическая кровь, Г-КСФ, химиотерапия, комбинированный протокол, исход трансплантации